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Microbiome community and complexity indicate environmental gradient acclimatisation and potential microbial interaction of endemic coral holobionts in the South China Sea

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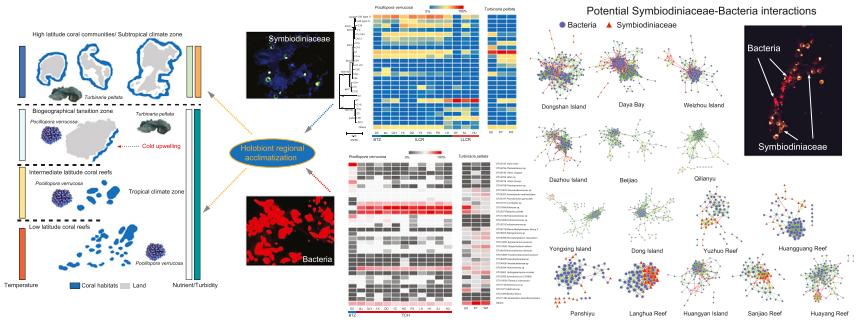
HIGHLIGHTS

- Coral microbial communities are linked to latitudinal/climatic environmental regime.
- Core microbiome abundance change may lead to microbiota function variation.
- Anthropogenic bacteria in core microbiome suggest large-scale human activity.
- Microbial α diversity is closely associated with co-occurrence network complexity
- Potential SBI may be driven by *Cladocopium*, γ-proteobacteria, and α-proteobacteria.

GRAPHICAL ABSTRACT



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ABSTRACT

Regional acclimatisation and microbial interactions significantly influence the resilience of reef-building corals facing anthropogenic climate change, allowing them to adapt to environmental stresses. However, the connections between community structure and microbial interactions of the endemic coral microbiome and holobiont acclimatisation remain unclear. Herein, we used generation sequencing of internal transcribed spacer (ITS2) and 16S rRNA genes to investigate the microbiome composition (Symbiodiniaceae and bacteria) and associated potential interactions of endemic dominant coral holobionts (*Pocillopora verrucosa* and *Turbinaria peltata*) in the South China Sea (SCS). We found that shifts in Symbiodiniaceae and bacterial communities of *P. verrucosa* were associated with latitudinal gradient and climate zone changes, respectively. The C1 sub-clade consistently dominated the Symbiodiniaceae community in *T. peltata*; yet, the bacterial community structure was spatially heterogeneous. The relative abundance of the core microbiome among *P. verrucosa* holobionts was reduced in the biogeographical transition zone, while bacterial taxa associated with anthropogenic activity (*Escherichia coli* and *Sphingomonas*) were identified in the core microbiomes. Symbiodiniaceae and bacteria potentially interact in microbial co-occurrence networks. Further, increased bacterial, and Symbiodiniaceae α-diversity was associated with increased and decreased network complexity, respectively. Hence, Symbiodiniaceae and bacteria demonstrated different flexibility in latitudinal or climatic environmental regimes, which correlated with holobiont acclimatisation. Core microbiome analysis has indicated that the function of core bacterial microbiota

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might have changed in distinct environmental regimes, implying potential human activity in the coral habitats. Increased bacterial α diversity may lead to a decline in the stability of coral-microorganism symbioses, whereas rare Symbiodiniaceae may help to retain symbioses. *Cladocopium*, γ-proteobacteria, while α-proteobacteria may have been the primary drivers in the Symbiodiniaceae-bacterial interactions (SBIs). Our study highlights the association between microbiome shift in distinct environmental regimes and holobiont acclimatisation, while providing insights into the impact of SBIs on holobiont health and acclimatisation during climate change.

1. Introduction

Coral reefs are diverse symbiotic ecosystems supporting an immense biodiversity while providing important ecosystems to a wide range of living organisms, including humans (Blackall et al., 2015; Hughes et al., 2017). However, coral reefs transitioning through the Anthropocene (Hughes et al., 2017) must contend with climate change, often undergoing configurational transformations caused by rising temperatures (Hughes et al., 2018; Stuart-Smith et al., 2018). The collapse of coral reefs that has occurred since the 1970s has largely been attributed to climate change, which has caused the coral cover to significantly decline by approximately 50–80% worldwide (Gardner et al., 2003; Bruno and Selig, 2007; Silverstein et al., 2015; Hughes et al., 2018). Biogeographical evidence showed that the global coral recruitment has declined by 85% throughout the tropics, but has increased by 78% in the sub-tropics (Price et al., 2019). Several studies have suggested that in some areas, such as eastern Australia (Booth et al., 2007; Figueira and Booth, 2010), Japan (Yamano et al., 2011), the western Mediterranean (Serrano et al., 2013), and the northern part of the South China Sea (SCS) (Tkachenko and Soong, 2017; Qin et al., 2019a, 2019b; Yu et al., 2019), sub-tropical coral reefs may be potential zones of refuge for tropical coral resisting the oceans' increasing temperatures (Beger et al., 2014). Studies on environmental tolerance and acclimatisation of dominant tropical and sub-tropical coral species are essential for assessing the environmental gradient acclimatisation of coral coping with climate change.

The environmental tolerance of coral are closely associated with coral host and microbiome, which includes endosymbiotic Symbiodiniaceae, bacteria, viruses, fungi, and archaea (Hernandez-Agreda et al., 2017; Brener-Raffalli et al., 2018; Osman et al., 2020). The coral host has a dynamic relationship with these diverse partners, collectively termed the coral holobiont (Brener-Raffalli et al., 2018). Certain corals adapt to different environmental stresses by restructuring their microbiome (particularly the structures of the Symbiodiniaceae and bacteria) (McDevitt-Irwin et al., 2017; LaJeunesse et al., 2018; Ziegler et al., 2019). Symbiodiniaceae, originated and diversified approximately 140–200 million years ago (coinciding with the adaptive radiation of calcifying corals during the middle Jurassic Period) (Simpson et al., 2011; LaJeunesse et al., 2018), have an abundant taxa with distinct eco-physiological and environmental tolerance (Lajeunesse et al., 2010; LaJeunesse et al., 2018; Chen et al., 2019a, 2019b; Qin et al., 2019a, 2019b). Several corals can adapt to new environmental regimes by shuffling or switching their Symbiodiniaceae (Baker et al., 2004; Fabricius et al., 2004; Stat et al., 2006). For example, the heat-tolerant *Durusdinium trenchii* is frequently involved in coral switching and shuffling adjustments in natural reefs (Pettay et al., 2015), contributing to the survival of the endangered *Orbicella faveolata* during thermal bleaching in the Florida Keys in 2015 (Manzello et al., 2018). In addition, bacterial communities influence the acclimatisation and adaptation of holobionts to environmental regime or stress responses (Ziegler et al., 2017; Ziegler et al., 2019). Several studies have reported that distinct bacterial communities can help coral respond to sea surface temperature (SST) variations (Ziegler et al., 2017; Brener-Raffalli et al., 2018; Osman et al., 2020), eutrophication (Ziegler et al., 2016; Ziegler et al., 2019), high turbidity (Ziegler et al., 2016), and low pH (Morrow et al., 2014). Corals also have symbiotic relationship with several beneficial bacteria, which can protect against pathogen invasion by secreting antibiotics (Ritchie, 2006; Rypien et al., 2010), inhibiting pathogen metabolism (Ritchie, 2006; Rypien et al., 2010), or consuming the pathogens itself (Welsh et al., 2015). Coral holobionts also have a core bacterial microbiome (present in 30 to 100% of the individuals of a coral species) that is associated with improved environmental adaptation and ecological function of the holobiont (Hernandez-Agreda et al., 2016; Brener-Raffalli et al., 2018). Core bacterial microbiomes have high specificities (Hernandez-Agreda et al., 2017; Hernandez-Agreda et al., 2018); thus, variation in the abundance of core microbiomes provides an insight into the adaptive strategy of coral holobionts in response to environmental stress in different habitats. Therefore, knowing the endosymbiotic Symbiodiniaceae and bacterial community structure, along with its variations, is critical to understand the coral holobiont acclimatisation toward distinct environmental regimes and the tolerance of corals to climate change.

Coral microbial communities not only affect the acclimatisation and immune response of the coral host (van Oppen and Blackall, 2019) but also interact with each other. The results of fluorescence in situ hybridisation (FISH) analyses showed that *Actinobacter* and *Ralstonia* have a close relationship with Symbiodiniaceae (Ainsworth et al., 2015). While other studies found that *Endozoicomonas* can protect Symbiodiniaceae from bleaching pathogens (Pantos et al., 2015; Neave et al., 2017). Altermonas and Cyanobacteria also provide nitrogen to the Symbiodiniaceae of coral larvae (Lesser et al., 2004; Ceh et al., 2013). Previous studies have reported that microbiomes are highly structured and form complex interconnected microbial networks in which microbes associate with each other either directly or indirectly through processes such as competition, facilitation, and inhibition (Barberán et al., 2012; Wagg et al., 2019; Chen et al., 2020). However, the drivers of potential Symbiodiniaceae-bacteria interactions (SBIs) and their association with microbial diversity remain unknown. This information helps to assess the symbiosis stability and acclimatisation of coral holobionts.

The SCS is on the northern edge of the 'Coral Triangle' (Spalding, 2001). Tropical atolls are widely distributed from the Zengmu Reef, (~4° N) near the equator, to the Dongsha Islands, (~20° N) in the northern SCS (Yu, 2012; Tkachenko and Soong, 2017; Chen et al., 2020). Several fringe reefs and coral communities are distributed along the northern edge of the SCS in sites including the Leizhou Peninsula (~20–21° N), Hong Kong (~21–22° N), and Dongshan Island (~23° N), and their distribution is controlled by the sub-tropical climate (Chen et al., 2009; Ng and Ang, 2016; Chen et al., 2019a, 2019b). The SCS covers 19 degrees of latitude, and corals throughout the SCS are exposed to environmental effects of not only different latitudinal gradients but also different climate zones (tropical and sub-tropical; Yu, 2012). Due to the Qiongdong cold upwelling, the eastern region of Hainan Island (18° 30′–20° 30′ N) exhibits sub-tropical features, including an increase in macroalgae cover (Chen et al., 2019a, 2019b) and sub-tropical coral species (Wu et al., 2013). Thus, the eastern region of Hainan Island can be defined as a biogeographical transition zone (BTZ), which is likely to become a refuge for tropical corals avoiding thermal stress (Beger et al., 2014). Moreover, investigating the genetic flow of coral hosts (*Porites lutea*, *Galaxea fascicularis*) highlights a high northern migration rate in the SCS (Su, 2017; Huang et al., 2018). Therefore, the SCS provides a suitable natural environment for studying the microbiome-driven latitudinal and climatic acclimatisation of coral holobionts associated with host's phylogeny.

This study aimed to explored the community structures of Symbiodiniaceae and bacteria associated with two endemic dominant coral species, namely *Pocillopora verrucosa* and *Turbinaria peltate*, collected from 15 coral habitats across 14 degrees of latitude. We characterised the latitudinal and climatic environmental factors to determine how microbial communities are linked to distinct environmental regimes and explained the associations between coral acclimatisation and microbial community shifts. Furthermore, network modelling inference was used to analyse the microbial co-occurrence network, which helps to explore potential SBIs and their key drivers in the coral holobionts. The results of this study will expand our understanding of the symbiosis stability and flexibility, acclimatisation, and potential microbial interactions of coral holobionts in response to global climate change.

2. Materials and methods

2.1. Sample collection, coral cover, and environmental characteristics

One hundred seventy-five tropical dominant *Pocillopora verrucosa* samples were collected from tropical coral habitats (TCH) and BTZ in the SCS (Table 1), and 44 sub-tropical dominant *Turbinaria peltata* samples were collected from three coral communities in the northern part of the SCS (Table 1). Only adult coral colonies were collected to control for the effect of age on microbiota composition (van Oppen and Blackall, 2019). Coral fragments (~2–3 cm^2) were obtained by hammer and chisel from a depth range of 2–15 m via SCUBA diving. The samples were cleaned in the boat with artificial sterile seawater (salinity: 35‰) to ensure they were not contaminated with free-living bacteria or Symbiodiniaceae. All fragments were transferred directly in preloaded 2 mL cryotubes containing 95% ethanol or 20% dimethyl sulphoxide (DMSO) buffer (Gaither et al., 2011), and stored at −20 °C until DNA extraction. Nucleic acids of holobiont microbiota were extracted using the DNeasy® plant mini kit (Qiagen, Hilden, Germany) and FastDNA® spin kit for soil (MP Biomedicals, France), according to the manufacturer's instructions. Genomic DNA was extracted from corals using a marine animal tissue genomic DNA extraction kit (Tiangen Biotech, Beijing, China), according to the manufacturer's instructions.

To determine the environmental influence of latitude gradient and climate (Osman et al., 2020), remote sensing MODIS-Aqua 4 km data of monthly SST (°C), chlorophyll *a* concentration (Chl *a*, mg/m^3), and the diffuse attenuation coefficient for downwelling irradiance at 490 nm (Kd, m^{-1}) were acquired from 15 sampling coral habitats in the SCS between 2009 and 2019 using the Giovanni Ocean Colour tool (https://giovanni.gsfc.nasa.gov/giovanni; Fig. 1). A one-way factorial analysis of variance (ANOVA) was used to analyse the monthly environmental data among different coral habitats (Fig. S1). Scheffe tests were used as post hoc multiple comparisons for further analysis of significant ANOVA results (Fig. S1). In addition, the description of geographic characteristics was based on those described by Chen et al. which include high latitude coral communities (HLCC), intermediate latitude coral reefs (ILCR), and low latitude coral reefs (LLCR) (Chen et al., 2019a, 2019b).

To determine how coral cover varied with latitude and climate, line intercept transect techniques were used, as implemented by the Australian Institute of Marine Science and recommended by the Global Coral Reef Monitoring Network (Zhao et al., 2013; Zhao et al., 2016; English et al., 1997). Benthic surveys were conducted at 14 coral habitats during the warm months (May to September 2017 and May to July 2018; Table S1). Sixty-five surveyed sites were visited at different depths and positions, ensuring representation of potential spatial community patterns of coral. The number of benthic survey sites for each coral habitat is presented in Table S1. Fifty metres of fibreglass measuring tape was fixed to the sea floor following the depth contour in each study site and an Olympus TG-4 Zoom digital waterproof camera was used to record videos; live cover of dominant coral was extracted from the videos (Chen et al., 2019a, 2019b). To determine the correlation between live coral cover and latitude gradient, linear regression analysis was conducted using GraphPad Prism 8.

2.2. Coral haplotype identification and analysis

We randomly selected 126 and 33 samples from *P. verrucosa* and *T. peltata*, respectively, for coral haplotype identification analysis (Table S2). The mitochondrial variable open reading frame (ORF) of *P. verrucosa* samples were amplified with FATP6.1 (5′-TTTGGGSATTC GTTTAGCAG-3′) and RORF (5′-SCCAATATGTTAAACASCATGTCA-3′) primers (Flot and Tillier, 2007; Brener-Raffalli et al., 2018) (Supplemental information S1). Since *T. peltata* phylogeny research is lacking, the molecular marker based on mitochondrial cytochrome B (cytB) genes was developed from the *T. peltate* mitochondrial genome (Shi et al., 2016). Primer 6.0 software was used to design specific primers. Ultimately, CytB-TURF (5′-ATGCCACTGCGCAAAGAGAA-3′) and CytB-TURR (5′-TTACGACGGACCATTCGCCT-3′), which exhibited the strongest specificity, were used to amplify cytB for haplotype identification (Supplemental information S1). Amplification products were directly sequenced on an ABI 3730XL DNA Analyser at Sangon Biotech (Shanghai, China). ORF and cytB sequences can be accessed in GenBank under accession numbers SUB7665150. Phylogenetic trees for these two endemic coral species were inferred using maximum likelihood (ML) and neighbour-joining (NJ) methods. The robustness of the tree was tested with 1000 bootstrap replicates. Haplotype network analysis for ORF and cytB sequences was conducted by DnaSp6 using their respective alignments, while degenerate base pairs and indels were ignored.

2.3. Microbiome identification and profiling

For all 219 endemic coral samples, amplicon sequencing was conducted for the two markers, respectively. The primers ITSintfor2 (5′-GATTGCAGAACTCCGTG-3′) (Lajeunesse and Trench, 2000) and ITS2-reverse (5′-GGGATCCATATGCTTAAGTTCAGCGGGT-3′) (Coleman et al., 2010) were used to conduct PCR amplification of the internal transcribed spacer region 2 (ITS2) of the Symbiodiniaceae rDNA. The precise methods of PCR and NGS can be found in Chen et al. (2020) and Chen et al. (2019a, 2019b), respectively. The bacterial community was analysed using 16S rRNA gene libraries generated with the 338F (5′-ACTCCTACGGGAGGCA GCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) primers targeting the highly variable V3-V4 regions (Liang et al., 2017). PCR was performed with ~10 ng of DNA, 1.6 μL (5 μm) primer, 0.4 μL TransStart Fastplu DNA Polymerase, 0.2 μL BSA, 4 μL 5 × FastPfu Buffer, 2 μL of 2.5 mM dNTPs, and ddH_2O to a total volume of 20 μL. GeneAmp® 9700 thermocycle controller was used to carry out PCR amplification with the following programme: 3 min at 95 °C, followed by 29 cycles of 95 °C for 30 s, 53 °C for 30 s, 72 °C for 45 s, and final extension at 72 °C for 10 min. The Qiagen Agarose Gel DNA Purification kit (Qiagen, Hilden, Germany) was used to purify PCR products, which were sequenced on an Illumina Miseq platform using the 2 × 300 bp mode according to standard protocols at Majorbio (Shanhai, China). The raw ITS2 and 16S reads were deposited into the NCBI Sequence Read Archive database (Accession Number: SRP267159 and SRP267164).

2.4. Bioinformatics processing

For Symbiodiniaceae ITS2 dataset analysis, quality control and sequence splicing of Illumina Miseq platform output data were conducted according to a commonly accepted protocol (Arif et al., 2014; Ziegler et al., 2017; Brener-Raffalli et al., 2018). Briefly, sequencing reads were de-noised using Trimmomatic software (Beger et al., 2014), and reads with a tail mass value of <20 were removed. Merging paired-end sequences and full-length ITS2 rDNA sequences were generated using MOTHUR. The quality-filtered reads were aligned to the ITS2 database using local BLASTN, and the parameters were set following the pipeline

Table 1
The samples of endemic dominant coral species from the South China Sea, including climate, sampling regions, the number of samples and sampling date information.

Climate	Regions	Sampling coral habitats	Coordinate	Coral species	The number of samples	Sampling dates (yyyy.mm.dd)
Biogeographical transition zones (BTZ)	Hainan Island	Dazhou Island	N18°42′-18°43′, E110°26′-111°30′	*P. verrucosa*	17	2019.07.10–2017.07.15
Tropic	Intermediate latitude coral reefs (IICR)	Beijiao	N17°06′-17°07′, E111°28′-111°31′	*P. verrucosa*	14	2019.08.02–2019.08.05
		Qilianyu	N16°54′-16°58′, E112°11′-112°20′		13	2019.08.06–2019.08.09
		Yongxing Island	N16°49′-16°50′, E112°20′-112°21′		15	2019.08.10–2019.08.13
		Dong Island	N16°39′-16°40′, E112°43′-112°44′		15	2019.08.15–2019.08.18
		Yuzhuo Reef	N16°18′-16°21′, E111°57′-112°5′		14	2019.08.20–2019.08.26
		Huangguang Reef	N16°9′-16°17′, E111°34′-111°49′		15	2019.08.28–2019.09.01
		Panshiyu	N16°2′-16°5′, E111°45′-111°50′		15	2019.09.02–2019.09.06
		Langhua Reef	N16°0′-16°5′, E112°26′-112°35′		15	2019.09.08–2019.09.11
	Low latitude coral reefs (LICR)	Huangyan Island	N15°06′-15°13′, E117°44′-117°50′		15	2017.07.13–2017.07.21
		Sanjiao Reef	N10°10′-10°13′, E115°16′-115°19′		14	2018.07.16–2018.07.15
		Huayang Reef	N8°51′-8°53′, E112°49′-112°51′		13	2018.07.18–2018.07.22
Subtropic	High latitude coral communities (HICC)	Dongshan Island	N22°33′-22°35′, E117°17′-117°25′	*T. peltata*	13	2018.07.01–2018.07.02
		Daya Bay	N22°34′-22°39′, E114°33′-114°39′		13	2018.07.13–2018.07.16
		Weizhou Island	N21°00′-21°04′, E109°04′ -109°08′		18	2018.08.22–2018.08.26

detailed in Chen et al. (2019a, 2019b). To accommodate the use of *ITS2* as a multicopy marker, we used sequence-based *ITS2* (sequences were present at a minimum cut-off of >5% for at least 1 of the 219 samples) analysis to identify dominant Symbiodiniaceae sub-clades (Ziegler et al., 2017; Chen et al., 2019a, 2019b). Moreover, Symbiodiniaceae α and beta diversities were analysed by operational taxonomic unit (OTU) clusters. MOTHUR was used to subsample qualified ITS2 sequences to 28,984 reads per sample, and sequences with a retention length of >90% were clustered into OTUs based on a similarity of 97% (Arif et al., 2014; Ziegler et al., 2017). The representative OTU sequences (most abundant) were aligned to a non-redundant ITS2 database, collated and published by Chen et al. using local BLASTN (Arif et al., 2014; Ziegler et al., 2017; Chen et al., 2019a, 2019b), while non-Symbiodiniaceae OTUs were removed (Table S5).

For bacteria 16S read analysis, PEAR software was used to merge the overlapping PE reads into 16S tags to allow for generation of the full-length 16S V3-V4 sequence (Zhang et al., 2014). Raw reads with homopolymer inserts and low quality scores (⟨20) were removed using UCHIME, and qualified reads were rarefied to an equal sequencing depth (30,323 reads per sample). After removing chimeric sequences, OTUs were clustered with a 97% similarity cut-off using UPARSE v7.1, and taxonomy of each 16S rRNA sequence was identified and classified using the Ribosomal Database Project (RDP v2.2) by setting a bootstrap confidence level of 0.7. The SILVA database release 132 was used for the 16S rRNA OTU alignment-based RDP classifier method using Quantitative Insights into Microbial Ecology 2 (QIIME2) (Bolyen et al., 2019). Mitochondria, chloroplast, and non-bacterial OTUs were removed from the dataset (Table S6).

2.5. Statistical analyses

Significant differences in Symbiodiniaceae and bacterial communities were tested by permutation multifactorial analysis of variance (PERMANOVA) with 9,999 permutation-based Bray-Curtis dissimilarity matrix. Non-metric dimensional scaling (nMDS) was used to visualise PEMANOVA results generated by Bray-Curtis distance in R (vegan package; Oksanen et al., 2015). The linear discriminant analysis effect size (LEfse) was used to identify shifts in the abundance of Symbiodiniaceae and bacterial OTUs among different coral habitats (LDA = 2.0; $p = 0.05$) in the Galaxy web application (https://huttenhower.sph.harvard.edu/galaxy). Correlations among environmental factors, abundance of microbiota and sampling regions were analysed by redundancy analysis (RDA) or canonical correspondence analysis (CCA), conducted in R using the vegan package (Oksanen et al., 2015); the significance levels were based on 1,000 Monte Carlo permutations. To define the contributions of environmental and geographical factors to the microbial community structure, a variation partitioning analysis (VPA) was performed using the varpart function of the vegan package (Oksanen et al., 2015). Redundancy of environmental variables was used to cluster variables by performing varclus in Hmisc R package (Harrell, 2008), which can limit co-linearity effects. QIIME2 was used to identify the bacterial core microbiome (Bolyen et al., 2019). OTUs consistently present in >80% of samples in one species were selected as conservative representative members of the core microbiome (Table S4; (Hernandez-Agreda et al., 2016; Hernandez-Agreda et al., 2017)). Moreover, phylogenetic investigation of communities by reconstruction of unobserved states 2 (PICRUSt 2) was applied to predict metagenomic functional content (KEGG-Pathway level 1, 2, and 3) of dominant bacterial community species (relative abundance >1%) from the 16S rRNA marker gene (Langille et al., 2013). Metagenome predictions were conducted using 'predict_metagenomes.py' and weighted nearest sequenced taxon index (weighted NSTI) was calculated for each sample (Langille et al., 2013). The LEfSe method was also used to identify significantly different metagenome functions of bacterial communities among coral habitats (LDA > 2.0; $p = 0.05$; Fig. S3 and Table S7).

To gain insights into the interactions between microbiota in the holobiont, a network modelling inference analysis was performed using the co-occurrence (CoNet) plugin for Cytoscape 3.7.2 (Faust et al., 2012). Briefly, dominant Symbiodiniaceae ITS2 sub-clades, and all bacterial OTUs as nodes, were used to construct the microbial

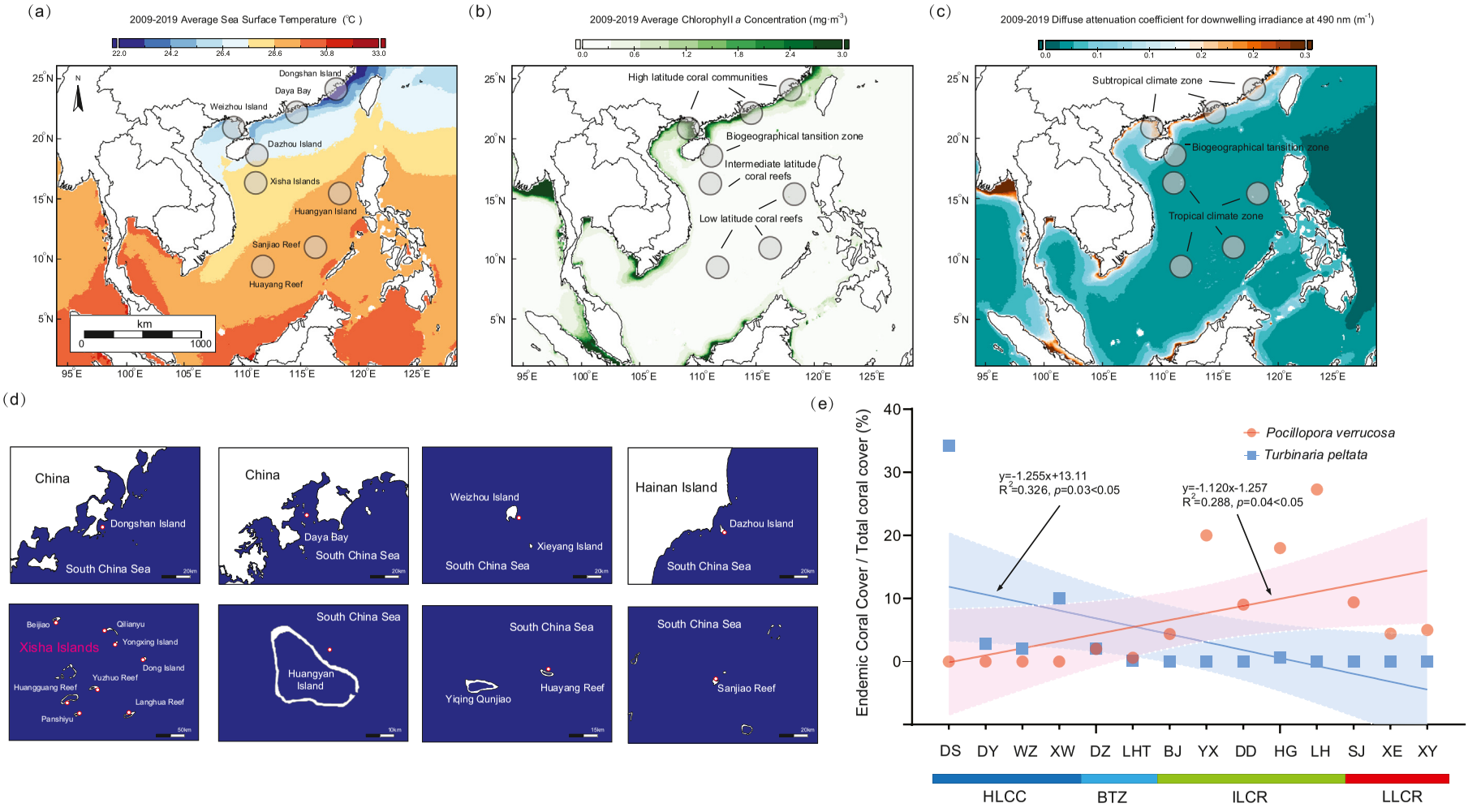

Fig. 1. Characterisation of environmental and two endemic dominant coral cover across the South China Sea for the period 2009–2019. The map shows the latitudinal gradient and climate zones shift of (a) annual average sea surface temperature (SST, °C), (b) chlorophyll *a* concentration (Chl *a*, mg·m^{-3}) and (c) diffuse attenuation coefficient for downwelling irradiance at 490 nm (Kd, m^{-1}), (d) including sampling sites. (e) Cover (%) of *Pocillopora verrucosa* and *Turbinaria peltata* among across high latitude coral communities (HLCC), biogeographical transition zone (BTZ), intermediate latitude coral reefs (ILCR) and low latitude coral reefs (LLCR) in the South China Sea.

interaction network. Taxa identified in at least two samples, and 20 reads were selected. Two measures of correlation and two measures of dissimilarity were used to estimate pairwise associations among Symbiodiniaceae and bacteria. Initially, 1,000 positive and 1,000 negative edges were retrieved as thresholds for four measures, and 1,000 normalised permutations and 1,000 bootstrap scores were generated to mitigate the combinatorial bias. Brown's method was used to merge and calculate the measure-specific *p*-value (Brown, 1975). After correcting for multiple comparisons using the Benjamini-Hochberg procedure (Benjamini and Hochberg, 1995), edges with merged *p*-values below 0.05 were retained. The interaction networks were visualised with Cytoscape 3.7.2 and the complexity of the interaction network was calculated as linkage density (links per OTU) among Symbiodiniaceae, bacteria, Symbiodiniaceae-bacterial only, or all Symbiodiniaceae and bacteria OTUs (Boucot, 1985; Wagg et al., 2019). In addition, The OTU abundance matrix for Symbiodiniaceae and bacterial communities was used to calculate α diversity indices, including Chao 1, Shannon diversity and Simpson evenness via MOTHUR version 1.30.1 (Figs. S4 and S5). Correlation between α diversity of microbiota and complexity of interaction networks were tested by linear regression using GraphPad Prism 8.

3. Results

3.1. Regional environmental differences and coral cover

Statistical analysis of the environmental index (NASA Giovanni satellite 2009–2019) revealed that the SST, Chl *a*, and Kd differed across coral habitats in the SCS (Figs. 1 and S1). The SST varied appreciably along the latitudinal gradient and decreased with increased latitude. Significant differences were observed in the SST among the HLCC (DS, DY and WZ, range from 23.06 ± 4.273C to 25.8 ± 4.300 °C), BTZ (DZ, 27.238 ± 2.342 °C), ILCR (BJ, QIY, YX, DD, HG, PS, and LH, range from 27.867 ± 1.796 to 28.424 ± 1.672 °C) and LLCR (HY, SJ, and HU, range from 29.018 ± 1.208 to 29.248 ± 0.958 °C; ANOVA, $F = 122.168$, $p < 0.001$; Figs. 1a, S1a and b), which agreed with previous biogeographical results (Chen et al., 2019a, 2019b; Chen et al., 2020).

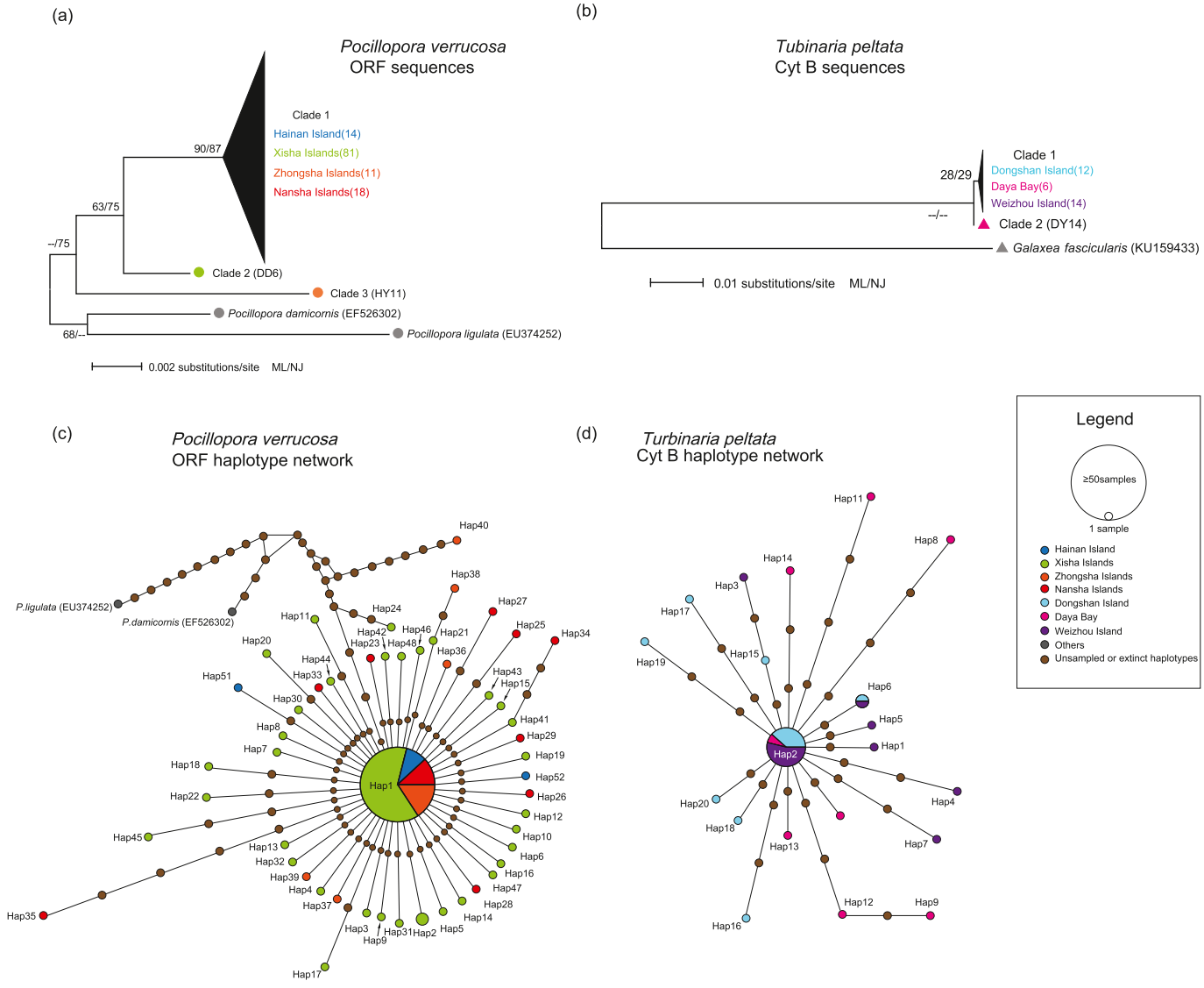

Fig. 2. Phylogenetic relationship and haplotype network *Pocillopora verrucosa* ($n = 126$) and *Turbinaria peltata* ($n = 33$) across the South China Sea. (a) Maximum likelihood (ML) tree of *P. verrucosa* mitochondrial variable open reading frame (ORF) sequences. (b) ML tree of *T. peltata* mitochondrial cytochrome B (cyt B) sequences. Values at nodes respectively represent ML and neighbour-joining (NJ) values >20%. The haplotype network constructed with (c) ORF sequences (d) cyt B sequences based on median-joining algorithm, respectively. Colours represent sampling sites, and brown circles represent unsampled or extinct haplotypes. Circle size is proportional to the haplotype frequency. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

However, the variation in the Chl *a* and Kd was associated with different climate zones in the SCS (Figs. 1 and S1). The Chl *a* of sub-tropical coral habitats (HLCC: from 1.274 ± 0.634 to 2.173 ± 0.502 mg/m^3) was significantly higher than that of the coral habitats in the BTZ and tropics (TCH; ILCR: range from 0.154 ± 0.086 to 0.300 ± 0.154 mg/m^3; LLCR: range from 0.118 ± 0.040 to 0.281 ± 0.360 mg/m^3; ANOVA, $F = 466.566$, $p < 0.001$; Figs. 1b, S1c and d). The values of Kd were homogeneous in the TCH and were < 0.01 (Fig. 1c). The Kd in the HLCC (range from 0.105 to 0.173 m^{-1}) was significantly higher than in the BTZ (0.019 ± 0.04 m^{-1}), and the Kd in both the HLCC and BTZ was higher than in the TCH (ANOVA, $F = 195.7$, $p < 0.001$; Figs. 1c, S1e and f).

The results of our ecological investigation showed that dominant tropical *P. verrucosa* coral cover decreased with increased latitude in the TCH (range from 0.6 to 27.3%; $R^2 = 0.288$, $p = 0.04 < 0.05$), a trend not observed in the HLCC (Fig. 1e). The biogeographic distribution characteristics of dominant sub-tropical *T. peltata* differed from those of *P. verrucosa* (varied from 0.2 to 34.2%; $R^2 = 0.326$, $p = 0.03 < 0.05$; Fig. 1c). The BTZ was the distribution boundary between these two endemic dominant coral species. The average percentages of *P. verrucosa* and *T. peltata* coral cover were 0.59–2.00 and 0.12–2.00%, respectively.

3.2. Coral host phylogeny and haplotype network

The phylogenetic analysis of *P. verrucosa* based on ORF revealed three potential clades corresponding to the coral host (clade 1, clade 2, and clade 3). However, two of the clades each contained only one sample, and most members of *P. verrucosa* belonged to clade 1 (Fig. 2a). A total of 50 haplotypes were found in the SCS, and most *P. verrucosa* haplotypes were closely related to, or derived from, Hap1 (Fig. 2c), which agreed with the phylogenetic results.

The ML/NJ phylogenetic tree based on the cyt B results revealed that *T. peltata* had two potential clades, although clade 2 contained only one sample (DY14). In addition, clade 2 was unstable in the phylogenetic relationship. Thus, significantly distinct clades of *T. peltata* in the SCS were not identified. Moreover, most individuals of the two endemic coral hosts were closely related to, or derived from, their own particular haplotype. Therefore, a direct association was detected between the latitudinal and climatic acclimatisation of coral holobiont and microbiome flexibility and plasticity.

3.3. Symbiodiniaceae community structure

ITS2 amplicon sequencing with MiSeq yielded 14,704,175 reads. After blasting and filtering the reads, 14,630,736 ITS2 reads were obtained. We identified *Cladocopium*, *Durusdinium*, and *Gerakladium* in the coral samples based on ITS2 sequence analysis after quality control (retaining ITS2 variants present with at least 5% abundance in at least one sample). At the sub-clade taxonomic level, 25 dominant Symbiodiniaceae types were identified, from which three genera, namely *Cladocopium* ($n = 18$), *Durusdinium* ($n = 6$), and *Gerakladium* ($n = 1$) were detected. For *P. verrucosa*, the relative abundance of *Durusdinium* was higher in the LLCR than in other coral habitats, except for the LH in the relatively lower part of the ILCR, while the D1 and D6 sub-clades were dominant in the LLCR (Fig. 3a). C42 (type1), C1d, C1·C45, C1, and C1ca were dominant members of the Symbiodiniaceae composition in the ILCR, however, the relative abundances of C1d and C1·C45 were low in the BTZ. For *T. peltata*, C1 was dominant in the Symbiodiniaceae composition among the DS, DY, and WZ from the HLCC (Fig. 3a).

One hundred and five Symbiodiniaceae OTUs were identified. To reduce the influence of intragenomic variation on Symbiodiniaceae beta diversity statistics, the OTU dataset was used for downstream analysis. The results of the nMDS analysis showed that variation in the Symbiodiniaceae community structure of *P. verrucosa* was associated with the shift in latitude gradient, and significant differences were apparent in community composition among the LLCR, ILCR, and BTZ (PERMANOVA, $F = 60.43$, $R^2 = 0.404$, $p < 0.001$; Fig. 3b). LEfSe analysis results indicated that 12 OTUs belonged to *Durusdinium* and that only one OTU of *Cladocopium* was enriched in the LLCR (LDA: 4.1–5.5; $p < 0.05$; Fig. 3d). The symbiont community of *P. verrucosa* had six enriched *Cladocopium* OTUs in the BTZ (LDA: 3.3–5.4; $p < 0.05$) and four *Cladocopium* OTUs in the TCH (LDA: 3.4–4.1; $p < 0.05$; Fig. 3d). In addition, there were significant differences in the *T. peltata* Symbiodiniaceae community structures among the DS, DY, and WZ (PERMANOVA, $F = 18.18$, $R^2 = 0.470$, $p < 0.001$; Fig. 3c), however, no OTUs with an LDA of >2.0 were identified in these coral habitats.

3.4. Bacterial community structure

After quality control of the 16S sequences, a total of 10,196,808 valid reads were identified. Notably, the effect of the variation in community structure of bacteria differed from that of Symbiodiniaceae in *P. verrucosa* (Figs. 3 and 4). The shift in the bacterial community was associated with changes in the climate zones (Fig. 4a). At the genus level, *Vibrio*, *Photobacterium*, *Pseudoalteromonas*, and *Endozoicomonas* were dominant in the BTZ bacterial community. *Curvibacter*, *Ralstonia*, and *Vibrionimonas* had a high relative abundance in *P. verrucosa* across all coral habitats in the TCH. In addition, the bacterial community structure of *T. peltata* was spatially heterogeneous, and *Ralstonia*, *Vibrionimonas*, and *Mollicutes* were dominant in the DS. *T. peltata* had the highest relative bacterial abundance (<1%) in the DY, while *Acinetobacter*, *Bradyrhizobium*, and *Sphingobacterium* were dominant in the WZ. The results of nMDS analysis showed there were significant differences between the *P. verrucosa* bacterial community structures of the BTZ and TCH (PERMANOVA, $F = 221.715$, $R^2 = 0.562$, $p < 0.001$; Fig. 4b).

Furthermore, the bacterial community structure of *T. peltata* differed significantly among the DS, DY, and WZ in the HLCC (PERMANOVA, $F = 7.541$, $R^2 = 0.268$, $p < 0.001$; Fig. 4c). LEfSe analysis results indicated that ten phylotypes were enriched in the dominant bacterial community of *P. verrucosa* (relative abundance >1%), which included *Vibrio*, *Endozoicomonas*, *Photobacterium*, *Thermus*, and *Pseudoalteromonas* (LDA: 4.2–5.3; $p < 0.05$; Fig. 4d). In the TCH, *Ralstonia*, *Vibronimonas*, *Curvibacter*, *Sphingomonas*, and *Brevundimonas* were enriched in the dominant bacterial community of *P. verrucosa* (LDA: 2.5–5.6; $p < 0.05$; Fig. 4d). In addition, only five phylotypes in *T. peltata* in the HLCC were enriched (Fig. 4e). *Ralstonia* and *Mollicutes* were enriched in *T. peltata* in the DS (LDA: 5.1–5.3; $p < 0.05$; Fig. 4e), and *Acinetobacter* and *Sphingomonas* were enriched in the WZ (LDA: 4.6–5.0; $p < 0.05$; Fig. 4e). However, we did not identify enriched bacterial phylotypes in the DY.

3.5. Environmental and geographic factors affecting microbial community structure

RDA/CCA was conducted to identify significant environmental and geographical factors affecting the communities of *P. verrucosa* and *T. peltata*. Temperature (SST variation-SSTv, maximum SST-MaxSST, minimum SST-MinSST, and average SST-SST), nutrition concentration (Chl *a* variation-Chl *a*v, maximum Chl *a*-MaxChl *a*, minimum Chl a-MinChl *a*, and average Chl *a*-Chl *a*), and turbidity (Kd)-associated factors were the important environmental variables chosen to study the community structures of Symbiodiniaceae and bacteria in *P. verrucosa* of the SCS (Fig. 5a and c). The relative abundances of Symbiodiniaceae and bacteria in the BTZ were positively correlated with higher levels of turbidity and SST variation, but negatively correlated with SST itself. Furthermore, geographical factors (latitude and longitude) also shaped the Symbiodiniaceae and bacteria communities (Fig. 5a and c). VPA was then conducted to further dissect the contribution of environmental and geographical factors to microbial community structure. These selected factors explained 45.2 and 51.8% of Symbiodiniaceae and bacterial community changes of *P. verrucosa*, respectively (Fig. 5a and c). Whereas the environmental and geographical factors accounted for only 12.3 and

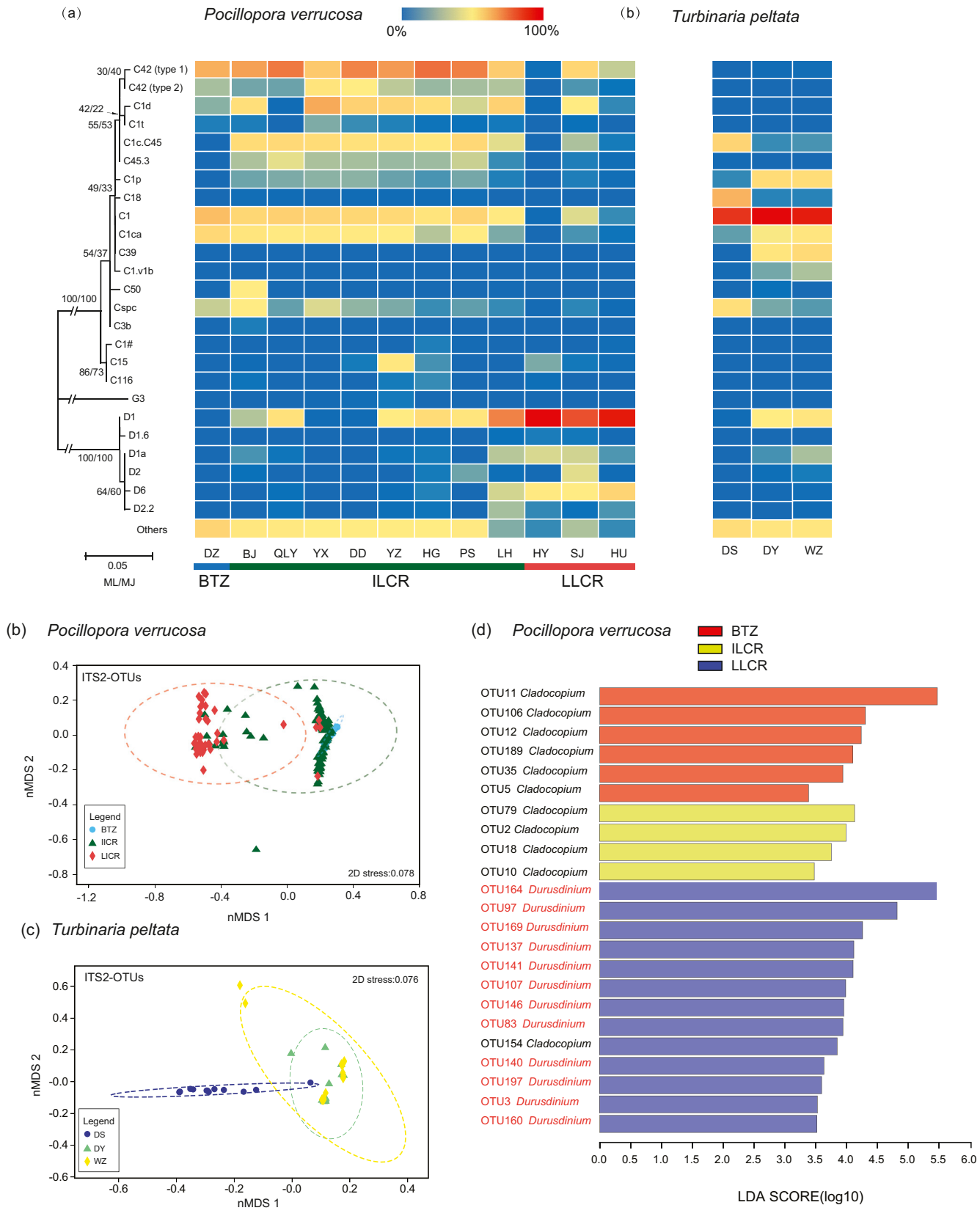

Fig. 3. Symbiodiniaceae community structure, relative dispersion and significant distinct taxa in the South China Sea. (a) The heatmap showed dominant Symbiodiniaceae community composition (sequences were present at a minimum cut-off of >5% at least one of the 219 samples) of *Pocillopora verrucosa* and *Turbinaria peltata*. The non-metric Multidimensional Scaling (nMDS) of Bray-Curtis distances of Symbiodiniaceae compositions associated (b) *P. verrucosa* samples in biogeographical transition zone (BTZ), intermediate latitude coral reefs (ILCR) and low latitude coral reefs (LLCR), and (c) *T. peltata* in Dongshan Island (DS), Daya Bay (DY) and Weizhou Island (WZ) from the high latitude coral communities (HLCC) based OTU analysis. Ellipses denote significant differences among distinct coral habitats ($p < 0.05$; PERMANOVA). (d) Enrichment Symbiodiniaceae genera with LDA scores of 2 or greater in symbiont communities of *P. verrucosa* among different coral habitats. Importantly, no Symbiodiniaceae OTUs enriched by *T. peltata* among different habitats in HLCC.

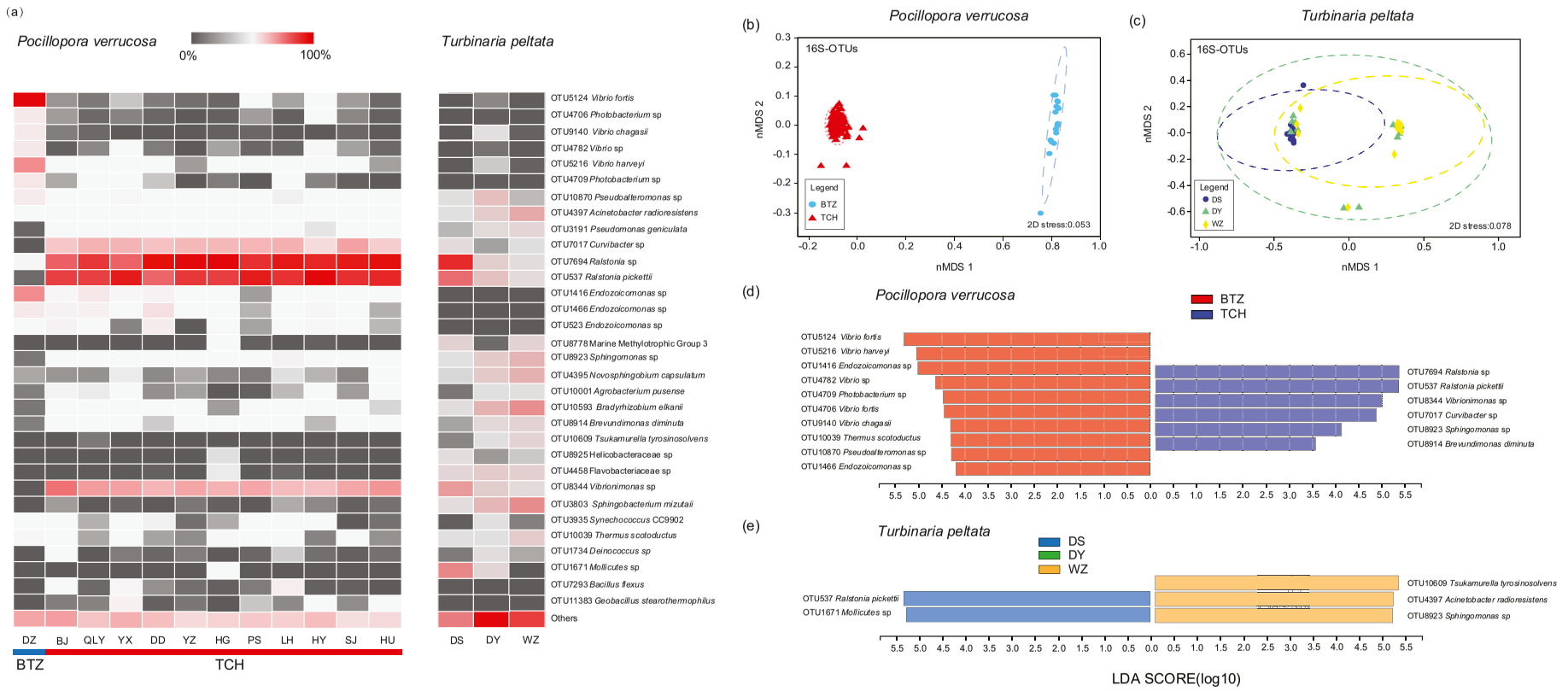

Fig. 4. Bacterial community structure, relative dispersion and significant distinct taxa in the South China Sea. (a) Taxonomic profile (OTU level) of the abundant bacterial community associated with *Pocillopora verrucosa* and *Turbinaria peltata*. The non-metric Multidimensional Scaling (nMDS) of Bray-Curtis distances of bacterial compositions associated with (b) *P. verrucosa* samples in biogeographical transition zone (BTZ) and tropical coral habitats (TCH), (c) and with *T. peltata* in Dongshan Island (DS), Daya Bay (DY) and Weizhou Island (WZ) from the high latitude coral communities (HLCC). Ellipses denote significant differences among distinct coral habitats ($p < 0.05$; PERMANOVA). (d) Enrichment bacterial OTUs with LDA scores of 2 or greater in symbiont communities of two endemic dominant coral species among distinct coral habitats.

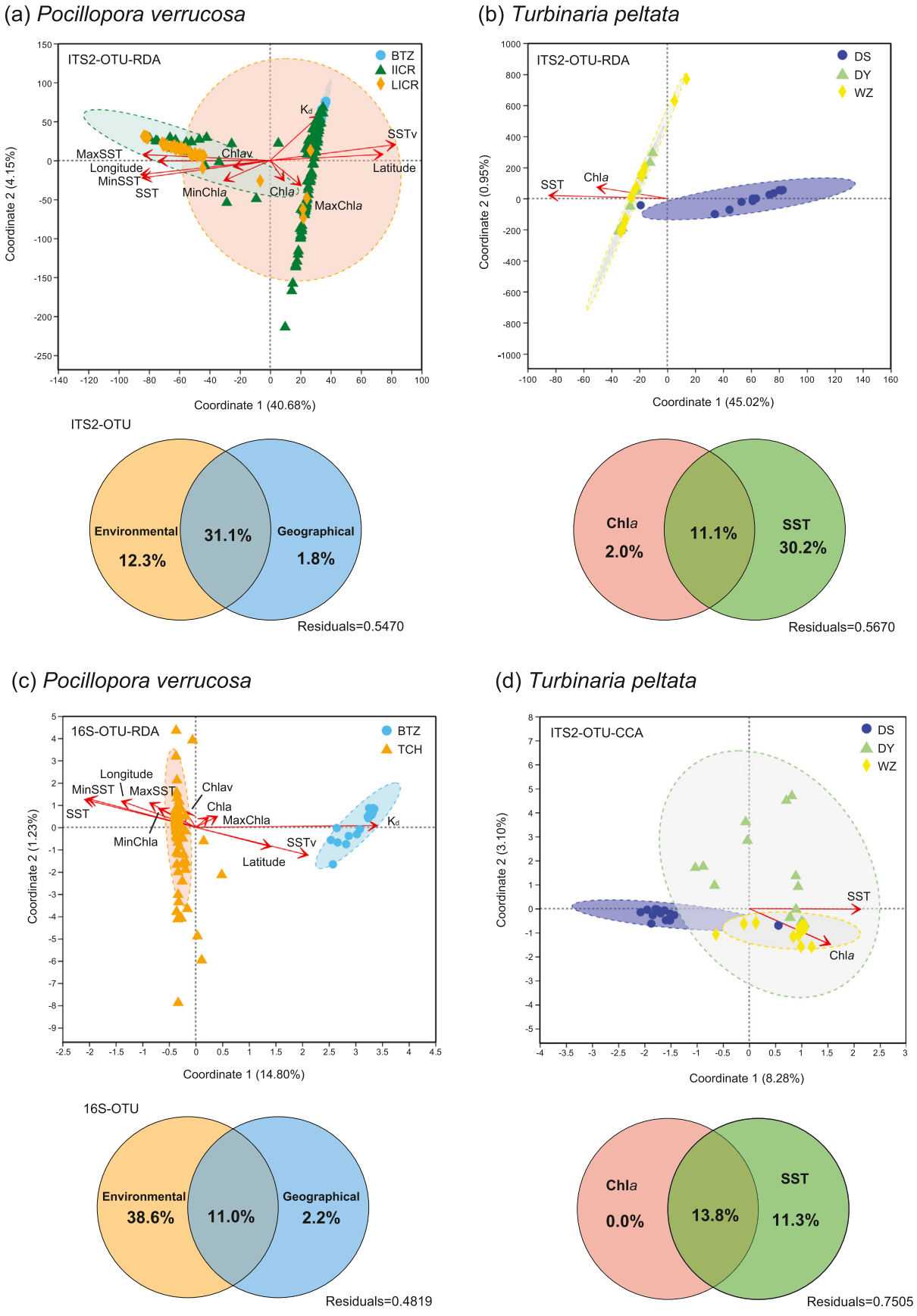

Fig. 5. Drivers of Symbiodiniaceae and bacterial communities in *Pocillopora verrucosa* and *Turbinaria peltata* samples in the South China Sea. (a, b) Redundancy analysis (RDA) or canonical correspondence analysis (CCA) showing environmental and geographical factors that affected Symbiodiniaceae community structure of and variation partitioning analysis (VPA) of the relative contributions of environmental and geographical variables to variation in Symbiodiniaceae Beta diversity. (c, d) RDA/CCA indicated bacterial community Beta diversity effected by environmental and geographical factors, and VPA of relative contributions of these factors (SST and Chl *a*) to variation in bacterial community structure.

1.8% of Symbiodiniaceae community changes, respectively; and 38.6 and 17.5% of the bacterial community shifts, respectively. In addition, the combined contribution of environmental and geographical factors explained 31.1 and 11.0% of Symbiodiniaceae and bacterial community changes, respectively. Taken together, these results indicate that combined effects of environmental and geographical variables affect Symbiodiniaceae and bacterial communities.

However, after removal of the redundant variables, only two environmental characteristics (average SST and average Chl *a*) were chosen for RDA/CCA of *T. peltata* in HLCC (Fig. 5b and d). The individual average SST contributed the highest percentage of variation in the *T. peltata* Symbiodiniaceae community structure (30.2%), while combined effects of average SST and Chl *a* contributed the highest percentage of variation to the bacterial community structure (13.8%).

3.6. Coral bacterial core microbiome

Bacterial phylotypes consistently present in >80% of the coral samples were members of a conservative representation of the core microbiome (Ainsworth et al., 2015; Hernandez-Agreda et al., 2018). Seven bacterial core OTUs identified were consistently associated with *P. verrucosa.* There were 15 core OTUs across the TCH, however, *P. verrucosa* had only seven core OTUs in the BTZ (Fig. 6 and Table S4). In addition, core microbiomes contributed between 83.6% of bacterial abundance DD, to 97.8% at SJ in the TCH (Fig. 6a), meanwhile the core microbiomes in the BTZ contributed only 0.6% of the bacterial abundance (Fig. 6a). The beneficial bacteria, *Ralstonia* (OTU537 and OTU7694) was the most abundant in *P. verrucosa* from the BTZ (0.32%) and TCH (33.22%; Fig. 6a). Meanwhile, potential anthropogenic *Escherichia coli* (OTU1477) were also identified in the *P. verrucosa* core bacterial microbiome (Fig. 6a).

For *T. peltata*, 17 consistently associated bacterial phylotypes were identified, which were present in >80% of *T. peltata* samples. These core bacterial members were all identified in the DS, DY, and WZ (Fig. 6 and Table S4). α-proteobacteria (41.1%) and γ-proteobacteria (17.6%) were dominant in the *T. peltata* core bacterial microbiome. Core bacterial microbiome phylotypes contributed between 9.5% of the bacterial abundance in the DS, to 31.2% in the WZ (Fig. 6b). In addition, four phylotypes were shared between the core microbiomes of the two corals, namely, *Sphingomonas* sp. (OTU8923), *Cutibacterium* sp. (OTU8436), *Acinetobacter* sp. (OTU3906), and *Rhodococcus erythropolis* (OTU6060). Beneficial bacterium of *Endozoicomonas* were not members of the core bacterial microbiomes of these two endemic dominant coral species.

3.7. Relationship between microbial diversity and network complexity

The co-occurrence network analysis showed that the complexity of the microbial network of *P. verrucosa* varied from 3.095 in the PS (in ILCRs) to 5.664 in the BTZ (Fig. 7a and Table 2). The average network complexity ranged from 3.993 in ILCRs to 3.982 in LLCRs (Fig. 7a and Table 2), suggesting that the co-occurrence networks in the TCH were simple compared with those in the BTZ. The degree of microbial network complexity in *T. peltata* was higher than in *P. verrucosa* (DS: 4.469; DY: 8.842; WZ: 8.500; Fig. 7a and Table 2).

To test assess the correlation between microbial α diversity and network complexity, Chao, Shannon, and Simpson evenness indices of Symbiodiniaceae and bacteria among each coral habitats were analysed (Figs. S4 and S5). Significant correlations between microbial α diversity and network complexity were observed (Figs. 7b and c). For Symbiodiniaceae, the Shannon ($R^2 = 0.504$, $p = 0.002 < 0.05$) and Simpson evenness ($R^2 = 0.230$, $p = 0.007 < 0.05$) indices were negatively correlated with network complexity (Fig. 7c), however, there was an insignificant correlation between Chao index ($R^2 = 0.216$, $p = 0.08$) and network complexity (Fig. 7c). Moreover, the Chao ($R^2 = 0.582$, $p < 0.001$), Shannon ($R^2 = 0.738$, $p < 0.001$), and Simpson evenness ($R^2 = 0.457$, $p < 0.001$) indices of bacteria were positively correlated with network complexity (Fig. 7d). Therefore, microbial network complexity increased with bacterial diversity and decreased with Symbiodiniaceae diversity.

Interestingly, potential multiple interactions between microbial members were identified in a co-occurrence network, especially the potential SBI (Fig. 7a, b and Table 2). In an analysis of the potential SBI correlations in *P. verrucosa*, 7–127 correlations were observed in the TCH, and the number of average correlations was 36, which was higher than in the BTZ (Table 2). Therefore, the potential SBI networks of *P. verrucosa* in the TCH were more complex than in the BTZ. Bacterial OTUs involving potential SBIs belonged to the classes γ-proteobacteria, α-proteobacteria, Actinobacteria, Oxyphotobacteria, Bacilli, Bacteroidia, and Dadabacteriia in the TCH, while only two bacterial classes, γ-proteobacteria and α-proteobacteria, were identified in SBIs in the BTZ (Fig. S6). *Cladocopium*, *Durusdinium*, and *Gerakladium* were involved SBIs in *P. verrucosa*, however, only *Cladocopium* was identified in potential SBIs in the BTZ (Fig. S6). In addition, in *T. peltata*, there were 19 potential SBI correlations were identified in the DS, 14 in the DY, and 14 in the WZ, accounting for fewer than those in *P. verrucosa* in the TCH, and a similar amount to those in the BTZ. The number of bacterial classes involved in SBIs in *T. peltata* was lower than in *P. verrucosa*. Five bacterial classes identified in SBIs belonged to γ-proteobacteria, α-proteobacteria, Actinobacteria, Bacteroidia, and Bacilli (Fig. S6). *Cladocopium* and *Durusdinium* were involved in SBIs in the DS and DY, respectively. Only *Cladocopium* was identified in potential SBIs in the WZ.

4. Discussion

4.1. Symbiodiniaceae community structure of coral holobionts is linked to latitudinal environmental regimes in the SCS

Results of the environmental factor analysis showed that variation in SST was associated with changes in the latitudinal gradient, which corresponded to the shifts in the Symbiodiniaceae community structures (Figs. 1 and 3). These results suggest that Symbiodiniaceae communities of *P. verrucosa* have strong geographical patterns in distinct latitudinal environmental regimes, which may be shaped by SST. Many other studies have similarly found that the spatial distribution pattern of the Symbiodiniaceae community is affected by temperature regimes (Kennedy et al., 2016; Reimer et al., 2017; Ziegler et al., 2017; Brener-Raffalli et al., 2018). For example, the dominant Symbiodiniaceae genus of *Porties* have shifted from *Cladocopium* to *Durusdinium* along the north-south gradient in the Red Sea (Terraneo et al., 2019). Furthermore, based on a molecular ecological study with the $PsbA^{ncr}$ gene marker, three distinct Symbiodiniaceae genotypes were reported to have a close association with thermal regimes in the northern and central Red Sea (Reimer et al., 2017). These geographical patterns have also been reported for other coral species in the SCS (*Galaxea fascicularis*, *Montipora*. sp); for example, a shift from a C3u sub-clade- to a C1 subclade-dominated community of *Acropora formosa* was found along the north-south latitudinal gradient (Chen et al., 2019a, 2019b; Tong et al., 2017). Moreover, *P. verrucose,* and its close relative, *Pocillopora acut* have highly stable Symbiodiniaceae communities in distinct environmental regimes (Sawall et al., 2015; Ziegler et al., 2015). However, Symbiodiniaceae communities of *P. verrucosa* have shown high flexibility across three latitudinal gradients in the SCS, which may imply that *P. verrucosa* holobiont in SCS adapt the strategy with flexibility of host-Symbiodiniaceae associations to facilitate acclimatisation to environmental regimes in distinct geographical regions. Specifically, *Durusdinium* was the dominant *P. verrucosa* symbiont community in the LLCR (Fig. 3a and d), which was involved in coral thermal acclimatisation via switching or shuffling of symbiont composition (Lajeunesse et al., 2010; LaJeunesse et al., 2018; Manzello et al., 2018), and improved heat tolerance of the coral host (Pettay et al., 2015;

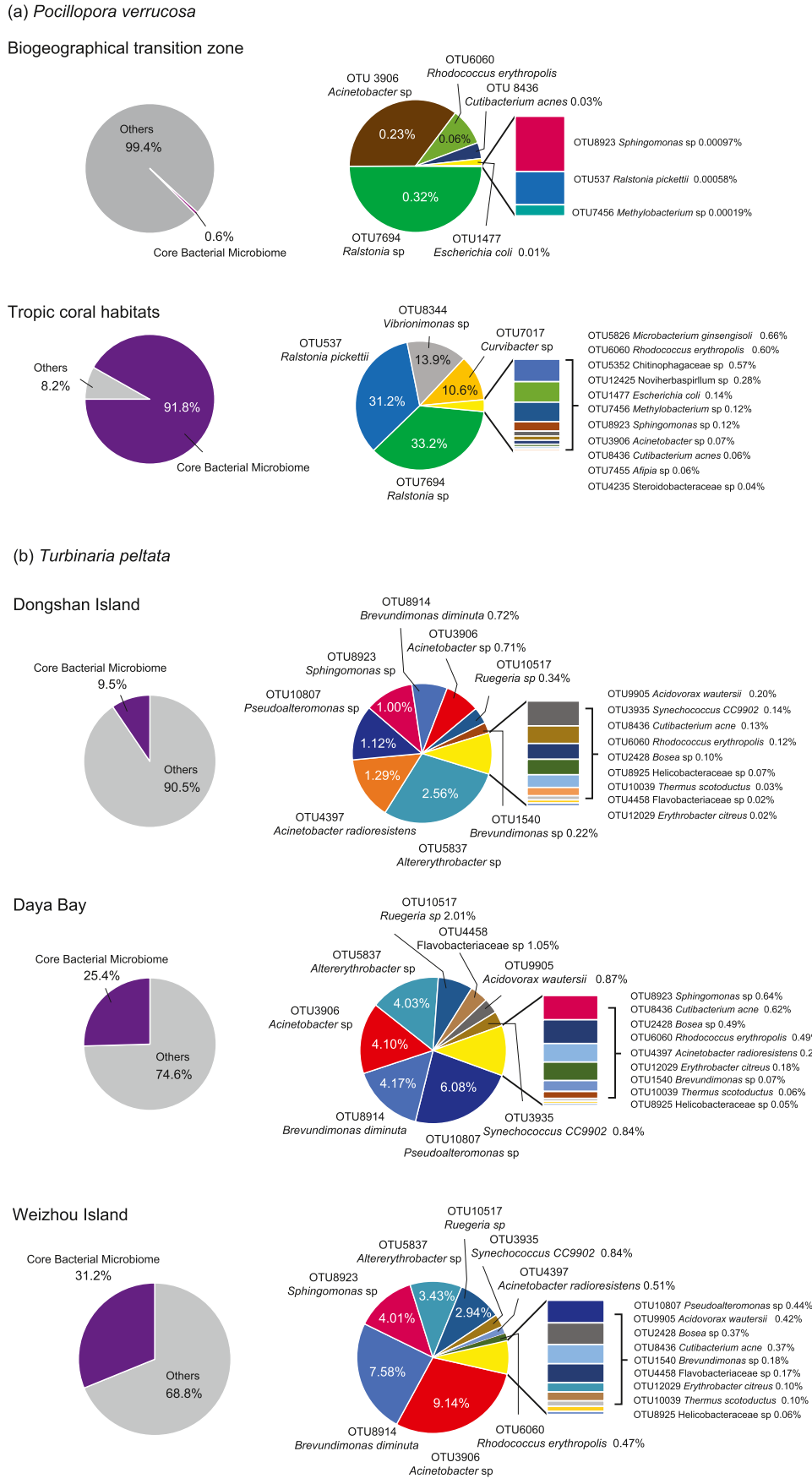

Fig. 6. The relative abundance and composition of core bacterial microbiome in (a) *Pocillopora verrucosa* and (b) *Turbinaria peltata* samples in the South China Sea. The pie chart on the left shows the percentage of core microbiome in coral bacterial community composition, and pie chart on the right illustrates the composition of core bacterial microbiome in coral holobionts. All values in panel represent the percentage of relative abundance of core bacteria in entire bacterial community.

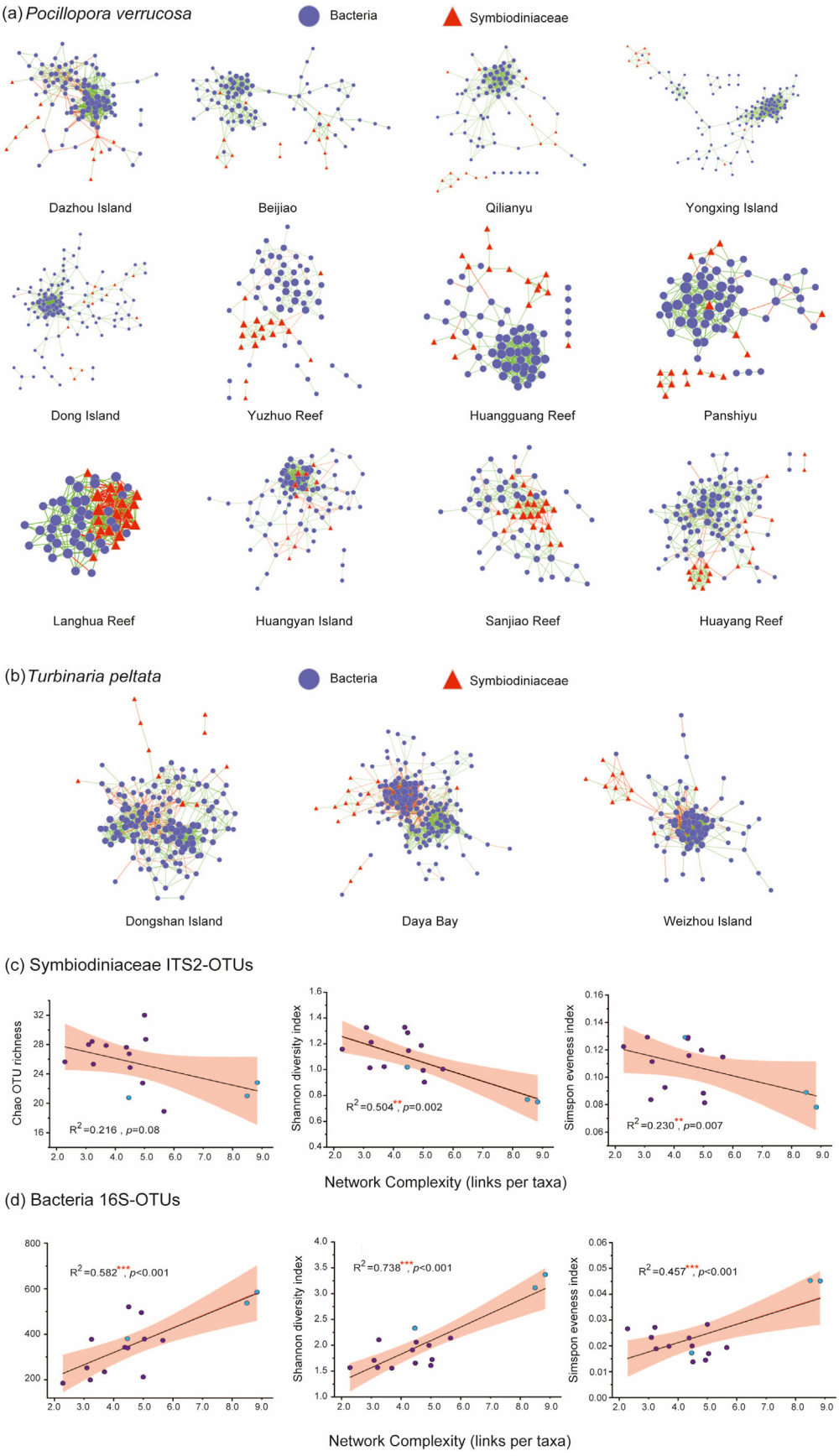

Fig. 7. The relationship between microbial interaction network complexity and alpha diversity of *Pocillopora verrucosa* and *Turbinaria peltata* in the South China Sea. (a) The microbial interaction network of *P. verrucosa* in biogeographical transition zone (BTZ) and tropical coral habitats (TCH). (b) The microbial interaction network of *T. peltata* in Dongshan Island (DS), Daya Bay (DY) and Weizhou Island (WZ) from the high latitude coral communities (HLCC). (c) Relationship between Symbiodiniaceae Alpha diversity (Chao, Shannon and Simpson evenness indexes) and microbial interaction network complexity. (d) Relationship between bacterial Alpha diversity and microbial interaction network complexity.

Table 2
The microbial network complexity and interaction correlations of *Pocillopora verrucosa* and *Turbinaria peltata* in the South China Sea.

Host	Sites	Network complexity	Node	Edge	Symbiodiniaceae-bacteria interaction			Symbiodiniaceae-Symbiodiniaceae interaction	Bacteria-Bacterial interaction
					Bacterial node	Symbiodiniaceae node	correlations	Correlations	Correlations
P. verrucosa	BJ	3.256	90	293	18	11	25	17	251
	QLY	4.383	81	355	18	6	18	33	304
	YX	4.508	126	568	6	4	7	21	540
	DD	4.939	131	647	8	7	11	22	614
	YZ	2.288	66	151	12	11	17	52	82
	HG	4.479	71	318	8	6	9	31	278
	PS	3.095	74	229	19	6	22	34	173
	LH	5	62	310	23	19	73	155	82
	HY	5.043	92	464	46	11	127	106	231
	SJ	3.212	66	212	16	13	52	96	64
	HU	3.692	107	395	17	14	25	72	298
	DZ	5.664	113	640	14	3	13	21	606
T. peltata	DS	4.469	143	639	13	12	19	57	563
	DY	8.842	177	1565	10	9	14	34	1531
	WZ	8.5	94	799	12	7	14	22	763

Silverstein et al., 2015; Boulotte et al., 2016). This result suggests that *P. verrucose* holobionts have acclimated to thermal regimes by establishing symbioses with heat-tolerant *Durusdinium* in LLCR. Thus, distinct thermal regimes may drive acclimatisation of *P. verrucosa* holobionts, and shape geographical distribution patterns of Symbiodiniaceae.

The dominant HLCC Symbiodiniaceae communities were homogeneous in *T. peltata*. C1 was the dominant type observed among different sampling sites in the HLCC (Fig. 3a). The results of LEfSe analysis suggest no enriched characteristics of *Symbiodiniaceae* OTUs in *T. peltata* among the DY, DS, and WZ. Moreover, HLCC is in a sub-tropical region of the SCS, with lower SST and higher nutrient concentrations and turbidity compared to BTZ and TCH (Fig. S2). Other studies have shown that C1 has a high photosynthetic efficiency and is acclimatised to environments with low SSTs and rich nitrogen-acquisition (Baker et al., 2013). Moreover, C1 is widely distributed in the northern region of the SCS, in Okinawa, and the Jeju Islands, which are characterised by a lower SST and higher levels of human activity (Reimer et al., 2006; Palmas et al., 2015; Ng and Ang, 2016; Chen et al., 2019a, 2019b). Thus, *T. peltata* may acclimate to sub-tropical regimes by consistently establishing symbioses with C1 in the HLCC of the SCS.

Taken together, Symbiodiniaceae community dispersion of coral holobionts correlated with latitudinal environmental regimes in the SCS; meanwhile, coral-Symbiodiniaceae symbioses acclimatisation was driven by SST with latitudinal gradient shift.

4.2. Bacteria microbiota of coral holobionts are associated with climatic environmental regimes in the SCS

Observed variations in the characteristics of the *P. verrucosa* bacterial community structure corresponded to changes in climate zones (Figs. 1 and 4). Specifically, the bacterial community structure was homogeneous in TCH, which agreed with results of a reciprocal transplantation experiment and investigation of bacterial spatial distribution reported in previous studies (Ziegler et al., 2016; Ziegler et al., 2019). Moreover, beneficial bacteria of *Ralstonia* (OTU7694 and OTU537) dominated the microbiota (and core bacterial microbiota) of *P. verrucosa* in TCH, with a relative abundance higher than that observed in BTZ (Fig. 4a and d). *Ralstonia* also plays important roles in ion-coupled transport and amino-acid metabolism in corals (Ainsworth et al., 2015). Meanwhile, FISH analysis has shown that *Ralstonia* live in the endosymbiotic bacterial communities of holobionts and form symbioses with Symbiodiniaceae (Ainsworth et al., 2015). Thus, *P. verrucosa* has low bacterial microbiota flexibility, and stable symbioses with beneficial bacteria of *Ralstonia* in TCH without SST variation or high turbidity. Alternatively, the bacterial community showed flexibility in BTZ with higher turbidity and SST variation (Fig. 4). Moreover, potentially pathogenic *Vibrio* (OTU5124, OTU9140, OTU4782, and OTU4709) exhibited higher relative abundance in *P. verrucosa*, and its presence may lead to an increase in the expression of genes associated with virulent factors in holobionts (McDevitt-Irwin et al., 2017; van Oppen and Blackall, 2019). Other studies have shown that the relative abundance of heterotrophic bacteria (e.g., *Vibrio*) increases in coral bacterial communities facing temperature stress, seawater pollution (McDevitt-Irwin et al., 2017; van Oppen and Blackall, 2019), or in those that are sick, or stressed (Bourne et al., 2008; Thurber et al., 2009; Meron et al., 2011; Lee et al., 2016). Thus, the increased relative abundance of opportunistic and pathogenic bacteria in coral holobionts may result from the effect of higher turbidity and SST variation.

Unexpectedly, *P. verrucosa* also harboured beneficial bacterium (*Endozoicomonas*, *Photobacterium*, and *Pseudoalteromonas*) with high relative abundances in the BTZ (Fig. 4). *Endozoicomonas* are located deep within coral tissues (Meron et al., 2011; Webster et al., 2016; Neave et al., 2017) and participate in the production of antimicrobial compounds (Bourne et al., 2008), helping prevent mitochondrial dysfunction in corals under stress (Ding et al., 2016). Similarly, the members of *Photobacterium* and *Pseudoalteromonas* also have antimicrobial activity against pathogens (Ritchie, 2006; McDevitt-Irwin et al., 2017; Kvennefors et al., 2011). This suggests that environmental regimes with higher turbidity and SST variation may lead to increased abundance of potential coral pathogens in BTZ. However, in response *P. verrucosa* may acclimate to a fluctuating environment in BTZ by increasing the abundance of beneficial bacteria with antimicrobial activity against coral pathogens, and which satisfy important metabolism requirements of coral holobiont (McDevitt-Irwin et al., 2017).

T. peltata bacterial communities exhibited high levels of flexibility with notable differences among the DS, DY, and WZ (Fig. 4), while no significant differences were observed in environmental factors among these sites, except Chl *a* (Fig. S2). Furthermore, potential beneficial (OTU537, *Ralstonia*) and harmful bacteria (OTU8923, *Sphingomonas* sp) were enriched by *T. peltata* in the DS and WZ, respectively (Fig. 4e; Ainsworth et al., 2015; Tang et al., 2019). Additionally, abundant background bacterial taxa were identified among the three sites. Taken together, these results may imply that bacteria microbiota was highly variable and flexible in response to slight environmental variations.

Differing from Symbiodiniaceae, bacteria microbiota of coral holobionts was associated with climatic environmental regimes in the SCS. specifically, the *P. verrucosa* bacterial community exhibited high flexibility in BTZ, yet was “inert” in TCH. Thus, *P. verrucosa* may acclimate to a fluctuating environment in BTZ by increasing the abundance of beneficial bacterium. In addition, bacterial microbiota of *T. peltata* has potential high variability and flexibility to acclimate to slight environmental variation in HLCC.

4.3. Core bacterial microbiota are affected by environmental regimes and potential anthropogenic disturbances in the SCS

Core bacterial microbiomes are of high ecological and functional importance in terms of holobiont fitness (Ainsworth et al., 2015; Brener-Raffalli et al., 2018; Hernandez-Agreda et al., 2018). However, the relative abundance of these microbiomes may be affected by distinct environmental regimes (Hernandez-Agreda et al., 2016; Hernandez-Agreda et al., 2017). In our study, the relative abundance of the core bacterial microbiome of *P. verrucosa* shifted from 96.5% in the TCH to 0.6% in the BTZ, suggesting that the members of the core microbiome shift from being predominant to rare bacterial species in the community (Fig. 6a). A study in the Great Barrier Reef and Coral Sea discovered eight highly persistent members of the core bacterial microbiome of *Pachyseris speciosa*, the abundances of which were distinct in areas facing anthropogenic stresses, such as sedimentation, runoff, and pollution that disturb the natural coral habitats (Hernandez-Agreda et al., 2016). This pattern is also present in mesophotic and shallow reefs (Hernandez-Agreda et al., 2016). Thus, the relative abundance of members of the core bacterial microbiome varies with environmental change (Hernandez-Agreda et al., 2016). Furthermore, the shift from dominance to rarity observed in our study may have been driven by higher turbidity and SST variation in the BTZ. Nevertheless, rare bacterial species are increasingly being recognised as crucial for hosts and ecosystems, with a disproportionately large role in biogeochemical cycles, and which may serve as potential drivers of microbiome function (Pedrós-Alió, 2012; Jousset et al., 2017). Moreover, under distinct environmental regimes, core bacterial species, such as rare members, may participate in the background of potential Symbiodiniaceae-bacteria-coral interactions and may provide the holobiont with access to otherwise unavailable nutrients and metabolic pathways (Ainsworth et al., 2015; Hernandez-Agreda et al., 2016; Hernandez-Agreda et al., 2018).

The coral holobiont core microbiome of *P. verrucosa* and *T. peltata* included several bacteria associated with human activity and pollution, which may indicate the spatial scale of the anthropogenic disturbance of coral holobionts. For instance, *E. coli* (OTU1477) was identified in the core bacterial microbiome of *P. verrucosa*, which may suggest that human activity has affected, or disturbed, coral in the BTZ and TCH. *E. coli* often reside in the gut of humans and other homeothermic animals and is a bacterial indicator associated with faeces and sewage (Alves et al., 2014). Hence, overfishing, active aquaculture, and the discharge of domestic sewage from fishing boats may account for the prevalence of *E. coli* in the SCS (Hughes et al., 2013). Furthermore, a potential coral pathogen, *Sphingomonas* (OTU 8923), was found to be common between the core bacterial microbiomes of the two endemic corals in this study. Unidentified species of *Sphingomonas* led to the "white plague" in the Florida Reef, causing infected coral show to exhibit an unusually rapid rate of tissue degradation (up to 2 cm per day) (Richardson et al., 1998). In addition, several members of *Sphingomonas* can degrade hydrophobic polycyclic aromatic hydrocarbons (PAHs) (White et al., 1996). In our study, *Sphingomonas* was widely present among the two endemic dominant corals, which may indicate that the corals were affected by PAHs in the SCS across 14 degrees of latitude. Meanwhile, previous studies have reported the detection of PAHs in coral mucous and tissues in the Nansha Islands (54 ± 9 ng/g dw), which are far from areas of heavy human activity (White et al., 1996; Han et al., 2020).

The core bacteria of *P. verrucosa* shifted in relative abundance from dominant to rare, suggesting that changes occur in the functions of the bacterial microbiome when coral holobionts acclimate to distinct environmental regimes (Hernandez-Agreda et al., 2016; Hernandez-Agreda et al., 2018). In addition, the core bacteria microbiome was characterised as being consistently present at a large spatial scale (Hernandez-Agreda et al., 2016; Hernandez-Agreda et al., 2017; Hernandez-Agreda et al., 2018). Therefore, the core microbiome included several bacteria associated with potential human activity (*E. coli*) and pollution (*Sphingomonas*), suggesting that potential anthropogenic disturbances have affected coral habitats across 14 latitudes in the SCS.

4.4. Microbial diversity and potential interactions may affect the health of coral holobionts

The impact of an individual species is intimately related to those of other species and results from a myriad of positive and negative, direct and indirect associations among the different species that as a whole drive ecosystem functions (Barberán et al., 2012; Wagg et al., 2019). In our study, *Cladocopium*, *Durusdinium*, and *Gerakladium* were potential SBI drivers, and the number of *Cladocopium* sub-clades involved in potential SBIs was higher than of other Symbiodiniaceae genera (Figs. 7and S6b). This result may be associated with the elevated level of functional diversity in *Cladocopium* (LaJeunesse et al., 2018). Indeed many studies have demonstrated that the members of *Cladocopium* are physiologically diverse with this genus accounting for the most ecologically abundant and broadly distributed among the Symbiodiniaceae (Pochon et al., 2006; Finney et al., 2010; LaJeunesse et al., 2018).

γ-proteobacteria and α-proteobacteria were involved in potential SBIs across all coral habitats (Figs. 7 and S6a) and may play important roles in the symbiosis between bacteria and Symbiodiniaceae. For example, beneficial bacterium *Endozoicomonas* belonging to γ-proteobacteria interacts with Symbiodiniaceae (Morrow et al., 2012; Pantos et al., 2015); while their involvement in sulphur cycling may help coral acclimate to environmental regimes by protecting Symbiodiniaceae from photosynthesis-derived oxidative stress (Sunda et al., 2002; Todd et al., 2010; Raina et al., 2013). Few studies have also reported on the potential interactions between α-proteobacteria and Symbiodiniaceae in coral holobionts. For instance, α-proteobacteria associated with dinoflagellates contain more gene transfer components (GTAs) than other proteobacteria, which may facilitate rapid evolution of surrounding microbiome strains (McDaniel et al., 2010; Webster and Reusch, 2017). Thus, we suggest that potential SBIs were primarily driven by *Cladocopium*, γ-proteobacteria, and α-proteobacteria and that potential SBIs are closely related to acclimatisation, health, and microbiome dynamics of coral holobionts.

Additionally, a positive correlation was observed between bacterial α diversity and microbial network complexity in the SCS (Fig. 7d). Meanwhile, increased bacterial diversity reduces the stability of the microbiome (McDevitt-Irwin et al., 2017; Zaneveld et al., 2017; van Oppen and Blackall, 2019), and stressors increase coral microbiome α diversity (McDevitt-Irwin et al., 2017). The coral holobiont microbiome is an open system (McDevitt-Irwin et al., 2017) that differs from that of the human gut (Lozupone et al., 2012). An increase in bacterial α diversity may indicate that the ability to regulate, or exclude, invasive microbial taxa is reduced, while opportunistic pathogenic bacteria from the surrounding environment contribute to this increase in microbial diversity (McDevitt-Irwin et al., 2017). Many previous studies have shown that corals have higher levels of bacterial α diversity in conditions with a lower pH (Meron et al., 2011), overfishing (Jessen et al., 2013), eutrophication treatments (Jessen et al., 2013), and algal contact (Pratte et al., 2018). In our study, elevated levels of bacterial diversity were closely associated with high levels of microbial network complexity, which may also indicate a decrease in coral microbial community stability. The high level of microbial network complexity corresponded to stronger interdependence among microorganisms, which may result in a reduced ability to resist disturbances to the microbial community (Barabási and Oltvai, 2004; Wang et al., 2014; Wang et al., 2016). Further, when a key microbial member disappears due to environmental stress, other related microorganisms may also lose their viability. Thus, our findings support those of previous studies suggesting that the increase in bacterial α diversity and microbiome complexity in coral holobionts may lead to declining stability of coral-microorganism symbioses.

Unlike that in bacterial α diversity, the increase in Symbiodiniaceae α diversity was associated with decreased microbiome complexity in coral holobionts (Fig. 7c), which may be associated with the rare Symbiodiniaceae biosphere (Boulotte et al., 2016; Ziegler et al., 2018). Moreover, a high level of host specificity is observed in the dominant Symbiodiniaceae community due to long-term co-evolution between coral and Symbiodiniaceae (Stat et al., 2006; Thornhill et al., 2006; Hume et al., 2015). Symbiodiniaceae communities are dominated by one to three sub-clades (Lajeunesse et al., 2010; Ziegler et al., 2017), with shuffling and switching events found to be rare during a short time frame (Boulotte et al., 2016). Although rare Symbiodiniaceae only account for <1–5% of the total symbiont community, they contribute to an increased level of diversity (Boulotte et al., 2016). In fact, robustness analyses have revealed that rare Symbiodiniaceae taxa contribute to host-symbiont community stability (Ziegler et al., 2018), and rare symbionts may improve the environmental resilience of the coral holobiont (Fabina et al., 2013; Boulotte et al., 2016; Ziegler et al., 2018). Therefore, we suggest that rare Symbiodiniaceae play an important role in maintaining the microbiome stability of coral holobionts by decreasing the level of microbial network complexity and improving the holobiont acclimatisation potential in distinct environmental regimes.

Cladocopium, γ-proteobacteria, and α-proteobacteria may have served as the key drivers in the potential SBIs of two endemic coral holobionts in the SCS. The diversity of Symbiodiniaceae and bacteria may directly correlate with the microbiome stability, coral holobiont health, and acclimatisation potential by affecting potential interaction network complexity.

5. Conclusions

Our study reveals distinctive characteristics of microbiome acclimatisation for two endemic coral species in the SCS. The Symbiodiniaceae community structure of tropical dominant *P. verrucosa* was linked to latitudinal environmental regimes in the SCS; meanwhile, the acclimatisation of coral-Symbiodiniaceae symbioses was primarily driven by SST with latitudinal gradient shifts. However, bacteria communities showed high flexibility in BTZ, which may be associated with high turbidity and SST variation. For sub-tropical dominant *T. peltata* the Symbiodiniaceae and bacteria community showed high stability and flexibility, respectively, which may imply that bacteria have higher plasticity than Symbiodiniaceae in the environmental regime with low SST, high turbidity and nutrition concentrations in HLCC. In addition, the relative abundance of the core bacterial microbiome of *P. verrucosa* shifted from dominant in TCH to rare in BTZ, suggesting that the functions of the core microbiome changed in distinct environmental regimes. Furthermore, bacterial taxa associated with anthropogenic activity (*E. coli* and *Sphingomonas*) were identified in the core microbiomes, which may indicate that large-scale potential anthropogenic activity, or disturbances, have affected coral habitats in the SCS. There were also close associations observed between microbiome α diversity and co-occurrence network complexity. Hence, increased bacterial α diversity may lead to a decline in the stability of coral-microorganism symbioses, while rare Symbiodiniaceae species may contribute to the retention of these symbioses. Moreover, *Cladocopium*, γ-proteobacteria, and α-proteobacteria may have served as the key drivers in the potential SBIs in the SCS. Our study highlights the association between microbiome shifts in distinct environmental regimes and holobiont acclimatisation, which provides insights into the impact elicited by SBIs on holobiont health and acclimatisation during climate change.

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CRediT authorship contribution statement

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Declaration of competing interest

The authors declare that they have no competing interests.

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